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Factors Controlling Attachment of Bryozoan Larvae: A Comparison of Bacterial Films and Unfilmed Surfaces

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Abstract. The effects of individual species of marine bacteria on the attachment of larvae of the bryozoan *Bugula neritina* were examined in the laboratory. Bacteria, grown to mid-exponential phase and allowed to adsorb to polystyrene petri dishes, attached at densities of 10^6–10^7 cells cm^{-2}. Bryozoan attachment assays (30 min) were used to compare the effects of adsorbed cells of three species of bacteria with unfilmed surfaces. Larvae permanently attached, at high percentages (65–94%), to unfilmed polystyrene, hydrophobic (*i.e.*, low wettability, low surface energy) control surfaces. This activity agrees with reports in the literature. Films of individual species of bacteria can influence bryozoan attachment. Three separate strains of the bacterial species *Deleya marina* inhibited attachment, but two other species of marine bacteria did not. Measurements indicated that all five bacteria tested differed in their cell-surface hydrophobicity, but that their films were similar in that they were all highly wettable (*i.e.*, high surface free energy). Our data indicate that factors in addition to substratum surface energy determine attachment of bryozoan larvae especially when bacterial films are present. Bacterial extracellular materials may be involved.

Introduction

Bryozoans are economically important marine fouling organisms (Ryland, 1976; Soule and Soule, 1977; Woollacott, 1984). The substratum preferences exhibited by their larvae range from the very specific to the very general (Ryland, 1974). Most bryozoans require a substratum that provides firm support for attachment, and many also prefer surfaces that have a smooth or glossy finish (Ryland, 1974, 1976). One critical characteristic in larval preference in settlement appears to be the surface free energy of the substratum (Mihm *et al.*, 1981; Woollacott, 1984). A surface with a low surface free energy has a low wettability and is hydrophobic, while a surface with a high surface free energy has a high wettability and is hydrophilic. Many bryozoan species prefer to settle and metamorphose on low surface free energy, hydrophobic substrata (Eiben, 1976; Loeb, 1977; Mihm *et al.*, 1981; Rittschof and Costlow, 1987a, 1987b, 1988).

Although a bacterial film is not essential for larval attachment (Ryland, 1976), microbial films on substrata influence the attachment of larvae from a number of different species of bryozoa (Miller *et al.*, 1948; Wisely, 1958; Crisp and Ryland, 1960; Ryland, 1974; Mihm *et al.*, 1981; Brancato and Woollacott, 1982). Mihm *et al.* (1981) demonstrated that bacterial films on polystyrene (a low surface energy substratum) caused the surface to become more wettable (higher surface free energy) and also decreased the attachment of larvae of the bryozoan *Bugula neritina*. However, Mihm *et al.* (1981) pointed out that the reduction in larval settlement could not be attributed to the change in surface free energy alone and suggested that *Bugula* larvae respond to two sensory stimuli: one for surface free energy, the second to some aspect of the bacterial-organic film. The latter stimulus could override the response to surface free energy alone (Mihm *et al.*, 1981). The response of bryozoan larvae to natural products that inhibit larval attachment may function by the same mechanism (Rittschof *et al.*, 1988).

It has been demonstrated that films, each composed of individual species of bacteria, can cause different attachment responses by spirorbid polychaete larvae (Kirchman *et al.*, 1982), barnacle cypris larvae (Maki *et al.*, 1988), and macroalgal swarmers (Thomas and Allsopp,

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1983). Perhaps the species composition of the bacterial films may also influence attachment by bryozoan larvae. We examined the following questions: (1) could films of individual species of bacteria elicit different attachment responses from bryozoan larvae? (2) would aging the bacterial films change the larval attachment response? (3) could larval attachment be correlated with surface free energy measurements using either bacterial cell-surface hydrophobicity or bacterial film wettability? We report here the results of laboratory experiments in which we tested the attachment responses of *Bugula neritina* larvae to bacteria irreversibly attached (Marshall *et al.*, 1971) to polystyrene surfaces. Our data indicate that the attachment of bryozoan larvae varied for both films of individual bacteria and films of different ages, and that larval responses were not correlated with measurements of either the bacterial cell-surface hydrophobicity or the bacterial film wettability.

Materials and Methods

All seawater used in larval attachment experiments was passed through a septic 100,000 Dalton ultrafiltration system (Millipore) and subsequently passed through two sterile filters (0.2 μm pore size, Millipore), placed one on top of the other. This seawater is referred to as filtered seawater (FSW).

Bacteria

Five cultures of marine bacteria were used in the larval attachment experiments. Four of the pure cultures were obtained from the American Type Culture Collection, Rockville, Maryland: *Deleya marina* 25374, *D. marina* 27129, *D. marina* 35142, and *Vibrio vulnificus* 27562. The fifth bacterium was isolate DLS1, a gram negative, polarly flagellated, oxidase positive, fermentative, rod-shaped bacterium that was isolated from the estuarine waters around the Duke University Marine Laboratory using Marine Agar 2216 (Difco, Detroit, Michigan). To preserve the bacteria and their respective surface characteristics, cultures upon receipt or isolation were frozen in vials with glycerol from which new stock cultures were periodically established.

Preparation of dishes with attached bacteria

Bacteria were allowed to attach to polystyrene petri dishes (Falcon 1006, 50 × 9 mm) following the methods outlined by Fletcher (1977). Cultures were grown to exponential phase at 26°C in Marine Broth 2216 (Difco, Detroit, Michigan) and harvested by centrifugation. Bacteria were washed, centrifuged, and resuspended in FSW (10^9 cells·ml^{-1}). Petri dishes were filled with 7 ml of the bacterial suspension and incubated at 26°C for 2.5–3.0 h. Dishes were rinsed by dipping them 10× in 500 ml of FSW. Bacteria still attached to the dishes were considered irreversibly attached (Marshall *et al.*, 1971). The dishes were then filled with 5 ml of FSW. Experiments were performed using either these dishes (for convenience termed Day 1) or with dishes in which the bacteria were aged *in situ* for 1 to 5 days. Aging of the attached bacteria in the dishes was accomplished through the following manner: every day after filling the Day 1 dish with FSW, it was emptied, and fresh FSW was added. Aged dishes were rinsed as above and refilled with 5 ml of FSW immediately prior to attachment assays.

Following the larval attachment experiments, two dishes of each bacterial treatment and control were fixed with formaldehyde (final concentration 1 to 2%, v/v) for quantification of attached bacteria using acridine orange and epifluorescence microscopy (Daley and Hobbie, 1975). These dishes did not receive any larvae. At least 300 bacteria were counted per dish and the number of cells expressed as bacteria per cm^2. Aged films were streaked on Marine Agar 2216 to check for contamination.

Larvae and attachment experiments

The larval bryozoan attachment assay was that previously described by Rittschof *et al.* (1988). *Bugula neritina* colonies were collected from the Duke University Marine Laboratory floating dock and from pilings of the north end of the Atlantic Beach, North Carolina, bridge. In the laboratory, colonies were maintained at 25° ± 3°C in the dark in aerated seawater and fed cultured diatoms (*Skeletonema costatum* Greville) at 100,000 cells ml^{-1} day^{-1}. *B. neritina* larvae were released in the morning in response to exposure to artificial light. Larvae (20–80) were collected in a 250–450 μl volume of seawater and pipetted into one of two dishes of each treatment (*i.e.*, bacterial films and controls) that already contained 5 ml of FSW. Repeated transfers of larvae resulted in a maximum of 160 larvae in any one dish. Assays were for 30 min at 22° ± 2°C. Timing of the assay began with the final larval addition and was terminated by the addition of a drop of formalin. Larvae adhering to the substratum and having no visible cilia (due to involution of the corona during metamorphosis, Zimmer and Woollacott, 1977; Woollacott and Zimmer, 1978) were counted as attached, while those that either were not adherent or had visible cilia were counted as not attached (90× magnification). Colonies used as a source of larvae were replaced when larval attachment to polystyrene fell below 50% in 30 min.

Experiments examined the effect of axenic films of bacteria and bacterial film age on the attachment of larvae. The first set of experiments used bacterial films of

different ages composed of either *Deleya marina* (ATCC 25374), *Vibrio vulnificus* (ATCC 27562), or isolate DLS1. The second set of experiments used bacterial films composed of either *D. marina* ATCC 25374, ATCC 27129, or ATCC 35142. Controls were FSW with no additions (polystyrene).

Frequencies of attached and not-attached individuals pooled from the two dishes were compared between treatments by G statistic (corrected for continuity) generated from a contingency analysis (Zar, 1984). The null hypothesis for the contingency analysis was that there was no significant difference between the control (polystyrene) and any one treatment. The family and individual level of significance in each group of comparisons was determined using Bonferroni's method for multiple comparisons (Seber, 1977).

Surface free energy measurements: bacterial cell-surface hydrophobicity and film wettability

Because the surface free energy has been shown to be important in the attachment of bryozoan larvae to a substratum, we determined both the cell-surface hydrophobicity of the bacteria in solution, using the adhesion to hydrocarbon method (Rosenberg *et al.*, 1980) and the wettability of films of attached bacteria derived from the same cultures, using measurements of air bubble contact angles (Fletcher and Marshall, 1982; Dillon *et al.*, 1989). Bacteria were grown to mid-exponential phase in Marine Broth 2216 (Difco, Detroit, Michigan) and harvested by centrifugation. Bacteria were washed once and resuspended in FSW or an artificial seawater, Nine Salt Solution (NSS) (Little *et al.*, 1986) to approximately 10^9 cells ml^{-1}.

To measure cell-surface hydrophobicity by adhesion to hydrocarbons (Rosenberg *et al.*, 1980), hexadecane (0.08, 0.16, 0.32, and 0.64 ml) was added to triplicate test tubes containing 4 ml of the bacterial solution (A_{400} = 1.3–1.5) and vortexed for 2 min. The phases were allowed to separate for 15 min and the absorbance (A_{400}) of the aqueous phase was measured spectrophotometrically. The results of the adhesion to hexadecane experiments are presented as the percent absorbance (A_{400}) left in the aqueous phase (bacteria with a high surface free energy and a hydrophilic cell surface would have a value of 100%).

Air bubble contact angle determinations (Fletcher and Marshall, 1982; Dillon *et al.*, 1989) were used as a measure of the wettability of unfilmed and filmed surfaces. Coupons (approximately 1 cm × 2 cm) of the polystyrene petri dishes (Falcon 1006) were placed in larger petri dishes (100 × 15 mm, Falcon 1029) and bacteria were allowed to attach to the coupons as for the petri dishes above. After attachment, coupons were retrieved with sterile forceps and rinsed as above and placed in another large petri dish containing FSW or NSS. For bubble contact angle measurements, the coupons were placed in a stage at the top of a chamber containing FSW or NSS. An air bubble, injected from a syringe (0.25 mm ID), was allowed to rise 6–7 mm to rest against the test surface. The average diameter of the bubble was 2.0 mm. Contact angles were measured directly using a Vernier microscope with a goniometer eyepiece. Results represent the mean of at least ten observations. For air bubbles where the air came in contact with the surface, errors were within 2° unless recorded otherwise; for air bubbles that did not make contact with the surface, indicating a high surface free energy, a value of <15° was recorded (Fletcher and Marshall, 1982; Dillon *et al.*, 1989). Coupons were then fixed with formaldehyde for quantification of attached bacteria as above. Comparisons of air bubble contact angle measurements on bacterial films were made to parallel measurements on muffled glass (500°C for 4 h) and polystyrene. *Bugula neritina* larvae have known attachment responses to these last two surfaces using the above attachment assay (Rittschof and Costlow, 1987a, b; 1988).

Results

Experiments were designed to examine the attachment of *Bugula neritina* larvae to bacterial films in the laboratory and to examine the larval attachment in relationship to estimates of surface free energies. These factors were hypothesized to be involved in the larval attachment response.

Bacterial densities

Bacteria adhered in densities of 10^6–10^7 attached bacteria per cm^2 both to the polystyrene petri dishes (Tables I, II) and to polystyrene coupons (Table III). Films with lower densities of bacteria (10^6 per cm^2) were not confluent but visually appeared randomly distributed rather than patchily. Films with higher densities of bacteria were confluent. Attached bacteria were undetected on the control polystyrene dishes indicating that the filtration of the seawater was effective in removing bacteria. The densities of attached cells on the aged dishes were lower than on Day 1 dishes, suggesting that some bacteria may have desorbed from the surface or lysed. Examination of the agar plates inoculated with bacteria from the aged dishes revealed only one colony type.

Larval attachment

The percentage of *Bugula neritina* larvae that attached to polystyrene control dishes in 30 min ranged between 66% and 93% (*e.g.*, Tables I, II). Bacterial films com-

Table I

Bugula neritina *larval attachment: data from experiments using films of different ages composed of three different species of bacteria,* Deleya marina, Vibrio vulnificus, *and isolate DLS1 attached to polystyrene petri dishes*

Treatment	No. of bacteria[a] ($\times 10^7$) cm^{-2} (+SD)	Total no. of larvae[b]	% larvae attached	G statistic[c] vs polystyrene	
				G No.	*P*
Polystyrene	nd	73	91.8		
D. marina					
Day 1	5.10 (0.18)	54	7.4	99.46	<0.001
Day 2	3.52 (0.30)	55	40.0	39.12	<0.001
Day 4	0.40 (0.06)	95	60.0	21.91	<0.001
V. vulnificus					
Day 1	4.59 (0.49)	67	79.1	3.65	NS
Day 2	2.60 (0.24)	263	97.7	3.58	NS
Day 4	1.47 (0.14)	179	97.2	2.25	NS
Isolate DLS1					
Day 1	1.73 (0.28)	74	81.1	2.78	NS
Day 2	0.86 (0.04)	94	87.2	0.48	NS
Day 4	0.53 (0.04)	140	96.4	0.37	NS

[a] Mean number of attached bacteria cm^{-2} from counts of two dishes using epifluorescence microscopy after staining with 0.01% (final concentration, w/v) acridine orange. Bacteria were grown to mid-exponential phase in Marine Broth 2216 (Difco, Detroit, MI) at 26°C, harvested by centrifugation, washed, and resuspended to 10^9 cells ml^{-1}. Dishes were exposed to bacterial solution for 2.5 to 3.0 h before being rinsed and used for experiments. Day 1 dishes were prepared the same day as the experiment, while Day 2 and 4 dishes were prepared 2 and 4 days prior to the experiment, respectively. FSW in these dishes was replaced daily after their preparation. nd = none detected.

[b] Total number of larvae in two dishes.

[c] Using Bonferroni's method of multiple comparisons, the family level of significance in the experiment was $\alpha = 0.05$ with an individual significance level of $\alpha/9 = 0.0056$ with 1 df where 9 is the number of comparisons. NS = not significant.

posed of either *Deleya marina, Vibrio vulnificus,* or isolate DLS1, and aged *in situ* for different lengths of time were tested for their effect upon attachment of *B. neritina* larvae. Films of *D. marina* of all ages significantly inhibited larval attachment compared to the polystyrene controls (Table I). Films of all ages composed of *V. vulnificus* and isolate DLS1 did not significantly inhibit larval attachment when compared to polystyrene controls (Table I). Similarly, larval attachment to films composed of *D. marina* was significantly lower than attachment to films composed of the other two bacteria on all days ($38.427 < G < 110.676$, $P < 0.001$, 1 df). Larval attachment on films composed of *V. vulnificus* or isolate DLS1 was only different on films aged for 2 days ($G = 11.826$, $P < 0.001$, 1 df). For all three bacteria, the larval attachment to Day 4 films was significantly higher than attachment to Day 1 films ($P < 0.001$, G statistic with 1 df).

In an experiment using films aged up to 5 days, larval attachment on *D. marina* films was again significantly inhibited when compared to the polystyrene control (control = 93.0% out of 214 total larvae attached, *D. marina* = 3.5–10.3% out of 58–86 total larvae attached, $P < 0.001$, G statistic with 1 df, family level of significance of 0.05, individual level of significance of 0.0056). Films of *V. vulnificus* (larval attachment = 82–98% of 70–108 total larvae) were not inhibitory ($P > 0.05$, G statistic with 1 df). Films composed of isolate DLS1 aged 3 days inhibited larval attachment (45.8% out of 59 total larvae attached, $P < 0.001$, G statistic with 1 df). Larval attachment on all other DLS1 films was similar to attachment to the control. Comparisons of larval attachment between films of different bacteria showed that attachment to films composed of *D. marina* was lower than on films of the other two bacteria on all days ($28.514 < G < 171.368$, $P < 0.001$, 1 df) and that attachment on isolate DLS1 was only different from that on *V. vulnificus* on films aged 3 days ($G = 58.393$, $P < 0.001$, 1 df). Larval attachment to older and younger films of *D. marina* was not significantly different ($P > 0.05$) while attachment to older and younger films of *V. vulnificus* and DLS1 were ($P < 0.005$ and $P < 0.024$, respectively, G statistic with 1 df). The data indicate that films of individual species of bacteria can affect the attachment of *B. neritina* larvae. Although there were statistical differences in larval attachment to older and younger films of the same species (with one exception, 3-day-old films of DLS1), in general, films of inhibitory species remained inhibitory and films of non-inhibitory species remained non-inhibitory compared to the controls. Experiments were conducted with three separate cultures of bacteria, all classified as *Deleya marina,* to determine if phenotypically similar bacteria could elicit different attachment responses from bryozoan larvae. All three cultures of *D. marina* significantly inhibited larval attachment com-

Table II

Bugula neritina *larval attachment: data from experiments using films of three strains of the marine bacterium,* Deleya marina, *attached to polystyrene petri dishes*

Treatment	No. of bacteria[a] ($\times 10^7$) cm^{-2} (+SD)	Total no. of larvae[b]	% larvae attached	G statistic[c] vs polystyrene	
				G No.	*P*
A.					
Polystyrene	nd	83	84.3		
D. marina					
ATCC 25374	2.86 (0.43)	66	4.5	105.42	<0.001
ATCC 27129	1.48 (0.28)	67	7.5	96.20	<0.001
ATCC 35142	1.76 (0.21)	73	15.1	78.73	<0.001
B.					
Polystyrene	nd	43	69.8		
D. marina					
ATCC 25374	2.52 (0.43)	72	2.8	60.77	<0.001
ATCC 27129	0.65 (0.09)	73	2.7	61.35	<0.001
ATCC 35142	1.79 (0.27)	66	1.5	62.33	<0.001

[a] The mean number of attached bacteria cm^{-2} from counts of two dishes determined using epifluorescence microscopy (see Table I footnotes).
[b] Total number of larvae in two dishes.
[c] Using Bonferroni's method of multiple comparisons the family level of significance in the experiment was $\alpha = 0.05$ with an individual significance level of $\alpha/3 = 0.016$ with 1 df where 3 is the number of comparisons.

pared to polystyrene controls (Table II). There were no significant differences in larval attachment to films composed of the separate cultures ($0.006 < G < 3.310$, $P > 0.05$, 1 df).

Surface free energy measurements: bacterial cell-surface hydrophobicity and wettability of films

The test to determine cell-surface hydrophobicity by adhesion to hexadecane showed that the three cultures of *D. marina* in solution were more hydrophobic (*i.e.*, had a lower surface free energy) than the other two bacteria (Fig. 1). However, air bubble contact angle measurements on films of the attached bacteria were all <15° even after aging the bacterial films. The air bubble did not come in contact with the surface and all films had a high surface free energy (Table III). Air bubble contact angles on polystyrene controls were 90° (low surface free energy) while those on glass baked at 500°C were also <15°.

Discussion

Bryozoan larvae have well-developed mechanisms for determining a suitable substratum, and these may be species specific (Ryland, 1976; Woollacott, 1984). The process begins with larvae gliding or crawling on a surface and testing it with cilia (Woollacott and Zimmer, 1978), and is often followed by a temporary attachment that employs an acid mucopolysaccharide adhesive (Loeb and Walker, 1977). Permanent attachment involves the eversion of the metasomal sac (Woollacott and Zimmer, 1978), which releases proteins that together with acid mucopolysaccharide provide the permanent adhesive (Loeb and Walker, 1977).

Previous investigations have illustrated that natural films of microorganisms can inhibit (Crisp and Ryland, 1960; Mihm *et al.*, 1981) or facilitate (Miller *et al.*, 1948; Wisely, 1958; Ryland, 1974; Mihm *et al.*, 1981; Brancato and Woollacott, 1982) the attachment of bryozoan larvae. Mihm *et al.* (1981) demonstrated that the presence of microbial films could make an unattractive substratum (*e.g.*, glass) attractive, and an attractive substratum (*e.g.*, polystyrene) unattractive. Our data demonstrate that, on a suitable polystyrene substratum, films composed of some bacteria significantly inhibit attachment of *Bugula neritina* larvae when compared to unfilmed controls. Other bacteria did not inhibit larval attachment. These data suggest that the species composition of a film may be important in the larval attachment response. Larval attachment to aged films of bacteria was generally higher than to freshly prepared films. The aging of the films resulted in a decrease in the density of attached bacteria (Table I) suggesting that bacterial density may be one important factor in the larval response to the film. However, in our experiments films composed of bacteria that were inhibitory to larval attachment remained inhibitory regardless of the age of the film. In contrast, films composed of bacteria that did not inhibit larval attachment remained uninhibatory in comparison with unfilmed polystyrene controls (Table I). Our experi-

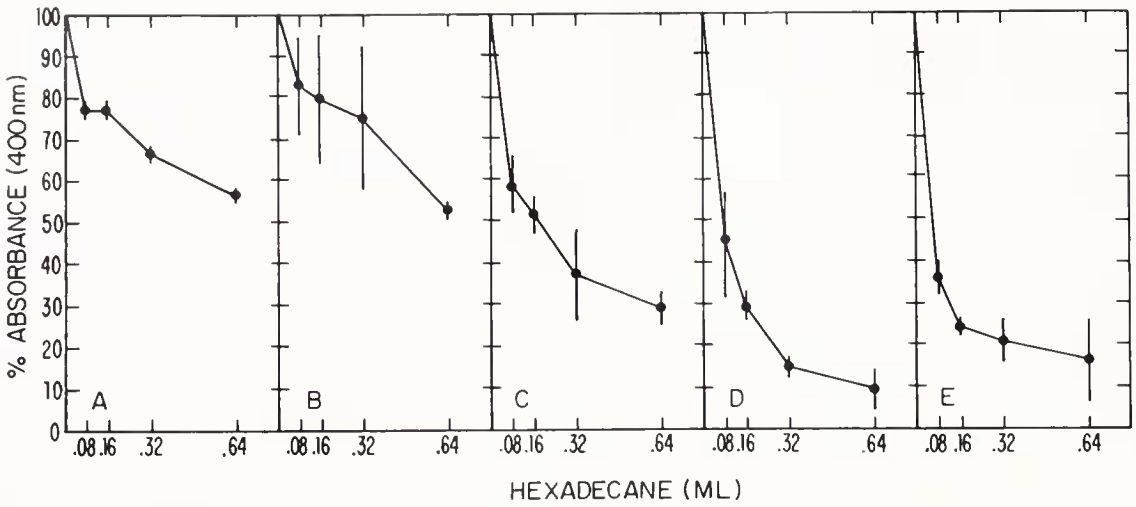

Figure 1. Affinity of mid-exponential phase bacterial cells to hexadecane as a function of hexadecane volume. Results are from three separate batch cultures and are expressed as percentage of the initial absorbance (A_{400}) remaining in the aqueous phase as a function of hexadecane volume. A. *Vibrio vulnificus* ATCC 27562. B. Isolate DLS1. C. *Deleya marina* ATCC 25374. D. *D. marina* ATCC 27129. E. *D. marina* 35142. Bars = standard deviation.

mental data indicate that the bacterial densities of 10^6 attached cells cm^{-2} were detectable by *B. neritina* larvae.

Because the surface free energy of the substratum is such an important factor in the attachment of bryozoan larvae, with larvae attaching in greater numbers to low surface energy, low wettability, hydrophobic surfaces, we used tests to measure both the cell-surface hydrophobicity and film wettability of the bacteria to determine if any correlations could be made between these measurements and larval attachment. The results of our cell-surface hydrophobicity experiments using the adhesion to hexadecane tests indicated that the three cultures of *Deleya marina* were the most hydrophobic (had the lowest surface free energy) of the five bacteria (Fig. 1). If cell-surface hydrophobicity of the bacteria was the dominant factor favoring bryozoan attachment to surfaces coated with bacteria, the larvae should have attached in greater numbers to the more hydrophobic (lowest surface free energy) bacteria (*i.e.*, the cultures of *D. marina*). However, films of *D. marina* were inhibitory to larval attachment when compared to both unfilmed polystyrene and films composed of *V. vulnificus* or isolate DLS1. Therefore, it appears that cell-surface hydrophobicity is not the dominant factor controlling the attachment of bryozoan larvae to surfaces possessing a bacterial film.

The measurements of the wettability of the bacterial film to estimate the surface free energy of the substratum may be more indicative of the surface sensed by a settling larva. Our data show that, regardless of the differences in the cell-surface hydrophobicity determinations of the bacteria, the films of all five bacteria had a similar high wettability and surface free energy (*i.e.*, they were hydrophilic) (Table III). Differences between cell-surface and colonial/film hydrophobicity have been previously reported for fish skin bacterial isolates and other bacteria (Sar, 1987; Sar and Rosenberg, 1987). The use of bubble contact angles permits the quantification of the wettabil-

Table III

Wettability measurements of bacterial films on polystyrene coupons using air bubble contact angles

Bacterium	Film age	Bubble contact angle[a]	No. of bacteria[b] ($\times 10^7$) cm^{-2} (+SD)
Deleya marina			
ATCC 25374	Day 1–Day 5	<15°	2.24 (1.37)–1.29 (0.95)[c]
ATCC 27129	Day 1	<15°	2.43 (0.14)
ATCC 35142	Day 1	<15°	2.65 (0.29)
Vibrio vulnificus	Day 1–Day 5	<15°	1.41 (0.09)–1.02 (0.13)[c]
Isolate DLS1	Day 1–Day 5	<15°	2.36 (0.09)–1.98 (0.12)[c]

[a] Bubble contact angle measurements of at least five air bubbles on surfaces of aged bacterial films on polystyrene coupons. Angles < 15° indicate that the bubble did not come in contact with the surface.

[b] Mean number of attached bacteria cm^{-2} from counts of at least five coupons using epifluorescence microscopy after staining with 0.01% (final concentration, w/v) acridine orange. Bacteria were grown and treated as in footnotes to Table I. Coupons were exposed to bacterial solution for 2.5 to 3.0 h before being rinsed and having their contact angles measured. Day 1 coupons were prepared the same day as the measurement, while Day 2, 3, 4, and 5 coupons were prepared 2, 3, 4, and 5 d before measurement, respectively. FSW for aged coupons was replaced daily after their preparation.

[c] Numbers represent the range of attached bacteria on coupons from Day 1 to Day 5.

ity (and therefore, the surface free energy) of a substratum that is normally in contact with a liquid medium (Loeb, 1985). Films of *D. marina, V. vulnificus,* and DLS1 transformed hydrophobic polystyrene (low surface free energy and wettability, contact angle = 90°) to a hydrophilic surface (high surface free energy and wettability, contact angle <15°) similar to glass, which is an unfavorable substratum for bryozoan larval attachment (Mihm *et al.,* 1981; Rittschof and Costlow, 1987a, b, 1988). If film wettability was the dominant factor that influenced bryozoan attachment in the presence of bacteria, then all the bacteria we used in our experiments should have elicited a similar unfavorable attachment response by the larvae. However, our data show that films of both *V. vulnificus* and isolate DLS1 (with one exception) were not inhibitory when compared to unfilmed polystyrene. These data support the hypothesis of Mihm *et al.* (1981) that bryozoan larvae possess a detection mechanism for an aspect of the bacterial-organic film other than its wettability (*i.e.,* surface free energy). Our data extend this hypothesis to individual species of bacteria.

Although Kirchman and Mitchell (1984) suggested that lectins on the surface of bryozoan larvae may mediate the choice of an attachment site by the larvae when bacterial films are involved, the actual sensory mechanism is unknown. However, the use of an adhesive in the temporary attachment of bryozoan larvae (Loeb and Walker, 1977) may create a situation analogous to that of attachment of barnacle cypris larvae. Crisp *et al.* (1985) have suggested that cypris larvae may not settle on, or permanently attach to, a substratum to which their temporary adhesive does not bind strongly. If bryozoan larvae function in a similar manner, then an explanation of our data may be that the temporary adhesive binds more strongly to the extracellular material of some bacteria than others. We are currently attempting to define the inhibitory factors involved in the attachment of these larvae to bacterial films.

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